

Serological techniques for the diagnosis of infectious diseases



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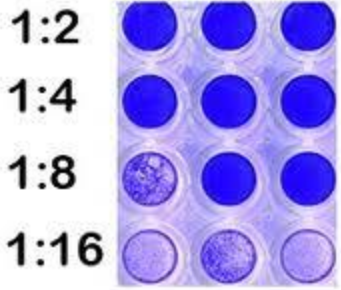
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Objectives

After the session, students should be able to:

- To list all the serological methods applied for the diagnosis of the infectious diseases “virology”.
- To differentiate between the different types of serological tools.
- To explain the theory behind the antigen-antibody reactions.
- To have full knowledge of serology and to be able to differentiate between advantages & disadvantages of the assays.
- To discuss and interpretation of the results.



What is Serology?

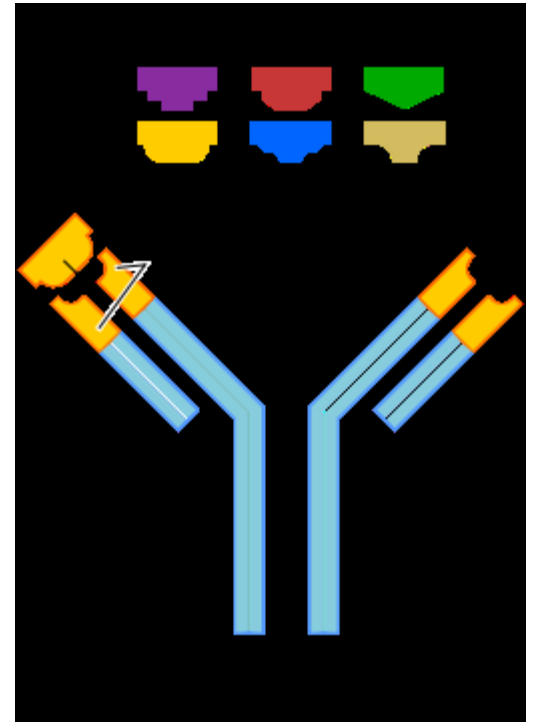
- **Serology** is the scientific study of plasma, serum and other bodily fluids.
- In practice, refers to the diagnostic identification of antibodies in the serum.
- The diagnostic microbiology “virology” laboratory is as critically important to the ID physician.

Serology test

- Serology testing allows the determination of immunity against different infecting organisms via the dosage of IgG.
- When the direct detection of a pathogen is not possible, detection of specific IgM antibodies or antigens may also help to diagnose an acute infection.

Antibody (Ab) Immunoglobulin (Ig)

- A large Y-shaped protein produced by B cells that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses.



Antibody Isotypes

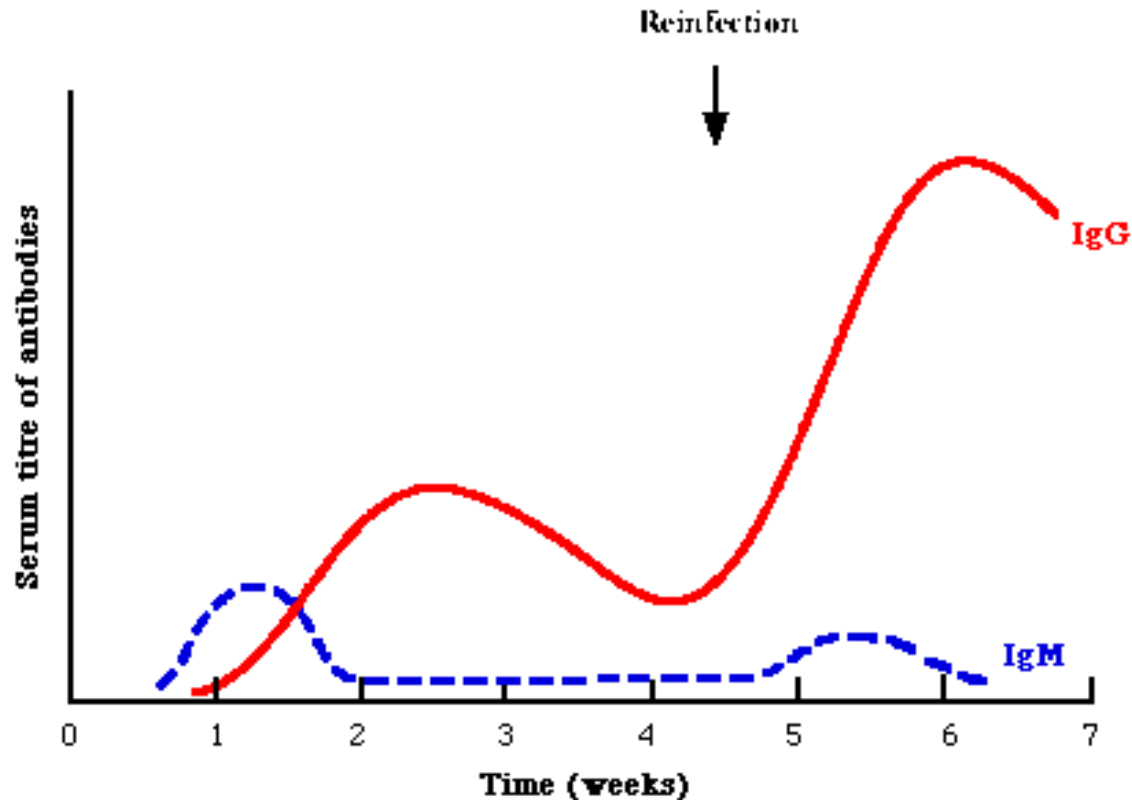
- Mammals there are five antibody isotypes known as **IgA**, **IgD**, **IgE**, **IgG** and **IgM**.
- They are each named with an "**Ig**" stands for immunoglobulin, another name for antibody, and differ in their biological properties, functional locations and ability to deal with different antigens.

Name	Type	Description	Antibody Complexes
<u>IgA</u>	Fecal-oral	Found in mucosal areas, such as the gut, respiratory tract and urogenital tract, and prevents colonization by pathogens. Also found in saliva, tears, and breast milk.	SI
IgD	Fecal - oral	Babies, young children	Colon
IgE	Food, Undercooked meat,	Sporadic, epidemic All ages	Colon
IgG	Fecal-oral	Young children	Colon
IgM	?	Children, adults, travelers	Colon

Antigen - antibody reactions.

- Antigen - antibody reactions are the methods by which antigens and antibodies are measured.
- Antibodies can be detected by various methods from the serum.
- Can be visualized in different ways according to the type of the antigen, conditions of the reaction and the medium the reaction takes place in.

Typical Serological Profile After Acute Infection



*Note that during reinfection, IgM may be absent or present at a low level transiently

Specimen collection

When sending specimens to the laboratory the following should be noted:

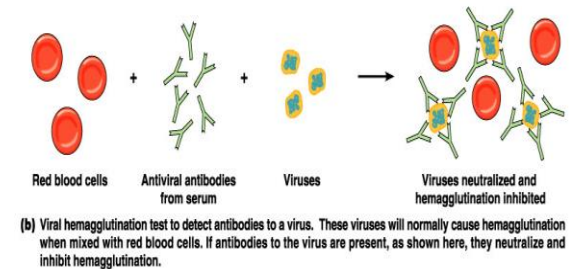
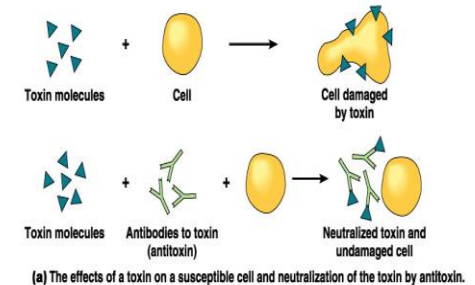
1. Specimen type” serum, whole blood, CSF,swab, fecal.ect.
2. Spesific co-agulants such as: EDTA, heaparin...
3. Distant clinics the serum or plasma should be separated within the hour, frozen and sent to the laboratory to arrive frozen.

Types of Serological tests

- Neutralization test
- Complement fixation test (CFT)
- Hemagglutination inhibition assay (HI test)
- ELISA's such as direct, indirect & sandwich
- Immunoprecipitation
- Immunoblotting (Western blot test)
- Immunofluorescence

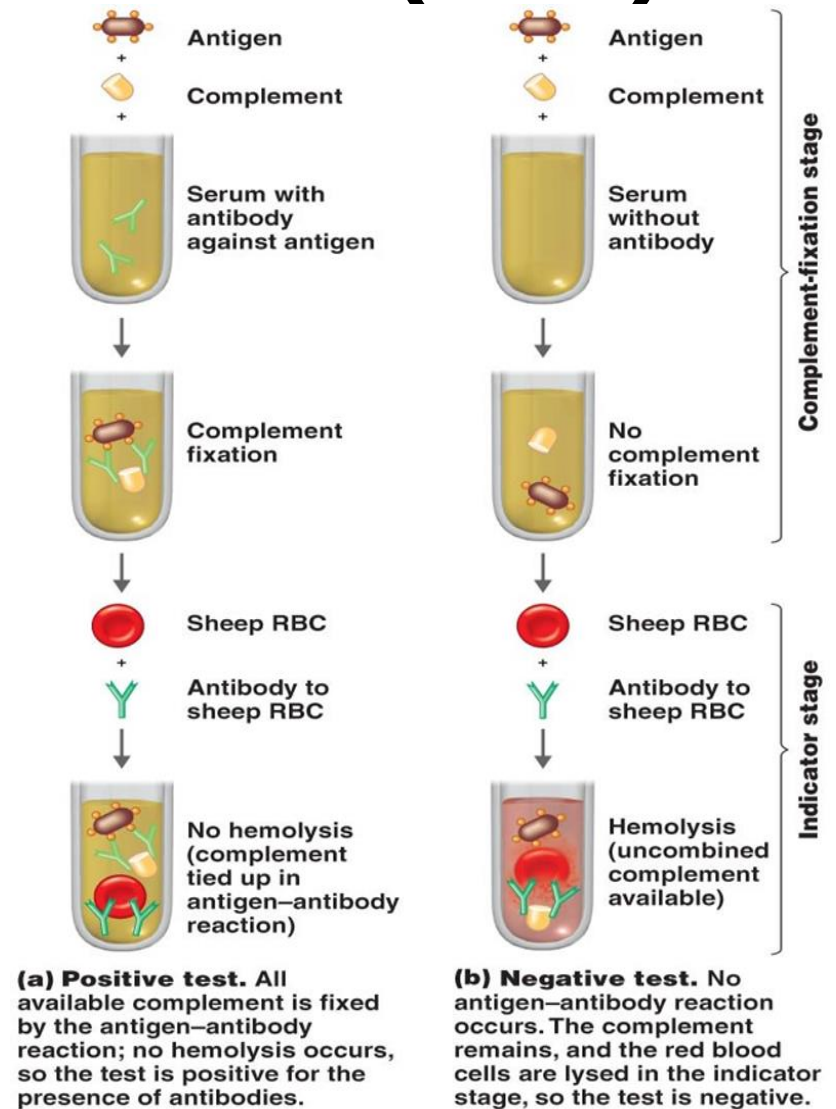
Neutralization test

- Neutralization test is defined as the loss of infectivity through reaction of the virus with specific antibody.
- NT used to quantify the titre of neutralising antibody for a virus.
- The presence of unneutralized virus may be detected by reactions such as CPE



Complement fixation test (CFT):

- CFT is convenient and rapid to perform, the demand on equipment and reagents is small, and a large variety of test antigens are readily available.
- CFT is not as sensitive as a neutralization test or hemagglutination inhibition test.



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Hemagglutination Inhibition test (HI):

- It measures the ability of soluble antigen to inhibit the agglutination of antigen-coated red blood cells by antibodies.
- Common non-fluorescence protein quantification assay specific "influenza".
- The assay takes ~1–2 hours to complete and results.
- Can differ widely based on the technical expertise.
- http://www.youtube.com/watch?v=aV8NPSiPh_4

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Anti-A **Anti-B** **Anti-D**

Name (Name/ Nom) _____
 Geb.Dat. (Date of Birth/ Date de Naissance) _____
 Datum (Date) _____
 Blut (Blood/Sang) _____

Kons.- Nr. (Unit No./ No.Poche) _____
 Blutgruppe (Blood Group/ Groupe Sanguin) **A POS**

Unterschrift (Signature) _____

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Direct Hemagglutination

Positive Reaction:

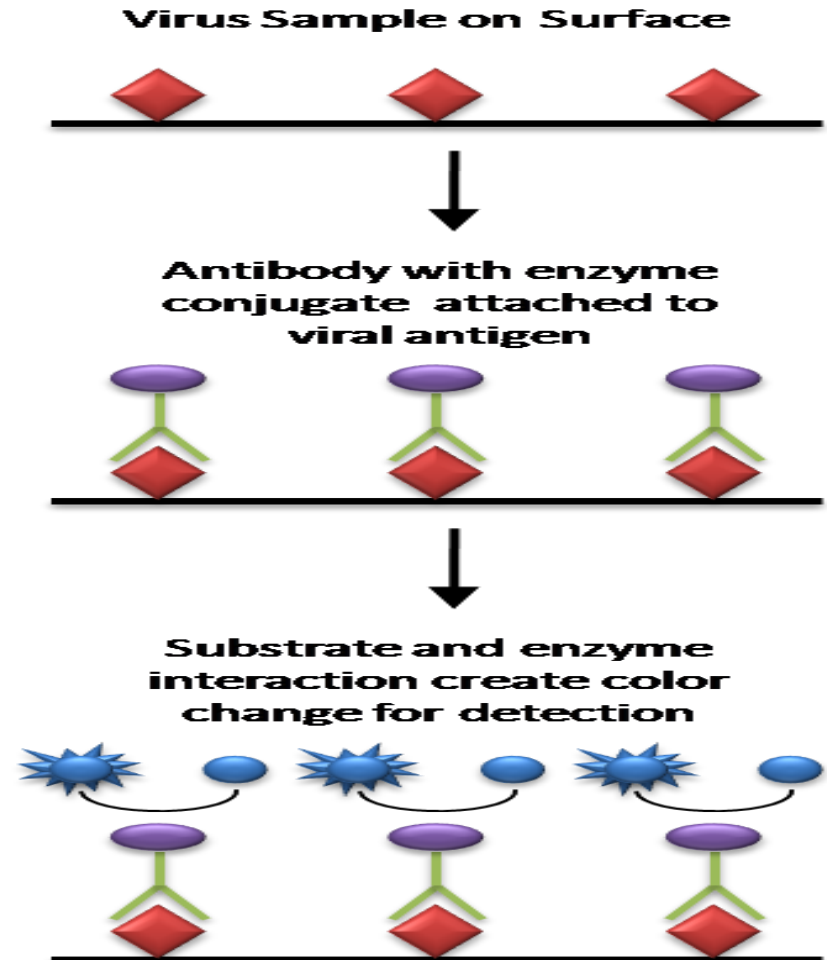
Hemagglutinating virus + Red blood cells = Agglutination

Negative Reaction:

Non-hemagglutinating virus + Red blood cells = No agglutination

ELISA

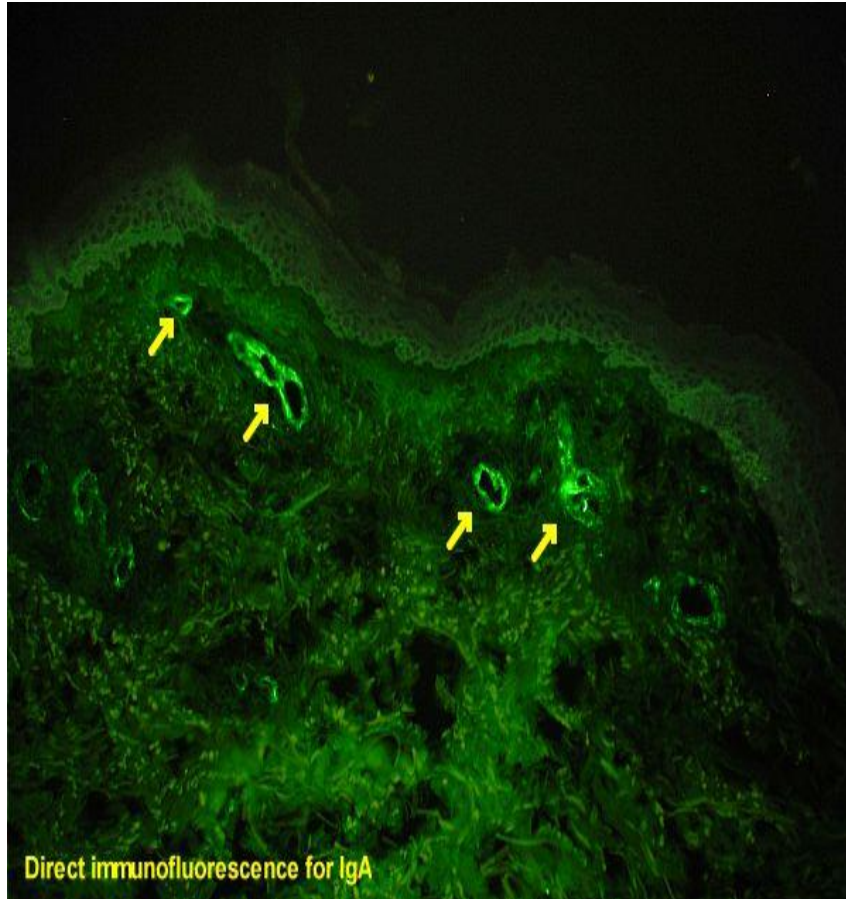
- Tubes coated with antigen.
- Antibody in serum attaches to antigen.
- Washing, add an anti-antibody tagged with enzyme alkaline phosphatase.
- Detectable color change occurs.
- <http://www.youtube.com/watch?v=tsRPW2QCh1Y>



Immunofluorescence

- A technique used for light microscopy with a fluorescence microscope.
- The specificity of antibodies to their antigen to target fluorescent dyes to specific biomolecule targets within a cell.
- Immunofluorescence can be used on tissue sections, cultured cell lines, or individual cells.
- limited to fixed (dead) cells when structures within the cell are to be visualized because antibodies cannot cross the cell membrane.

Types of immunofluorescence



- **Primary**, or direct, immunofluorescence uses a single antibody that is chemically linked to a fluorophore.
- **Secondary**, or indirect, immunofluorescence uses two antibodies

TCID50 (Tissue culture Infectious dose 50)

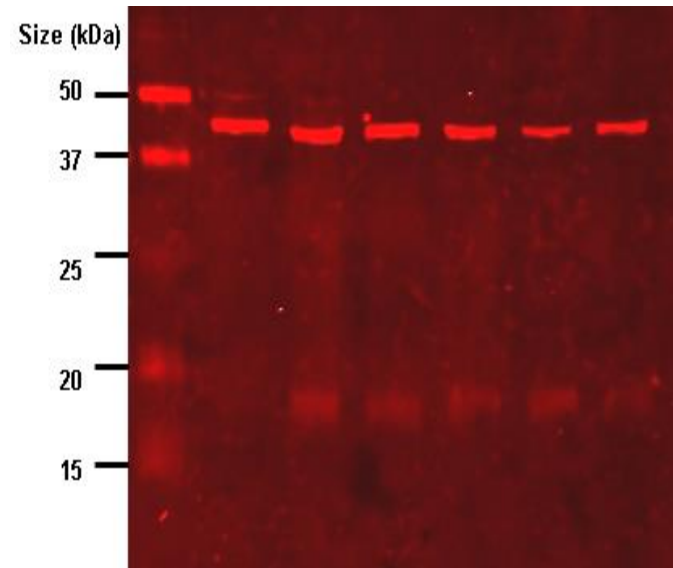
- The quantity of “virus” in a specified suspension volume (e.g., 0.1 ml) that will infect 50% of a number (n) of cell culture microplate wells, or tubes, is termed the “Tissue Culture Infectious Dose 50 (TCID50)”.

- The focus forming assay

FFA employs immunostaining techniques using fluorescently labeled antibodies specific for a viral antigen to detect infected host cells and infectious virus particles before an actual plaque is formed.

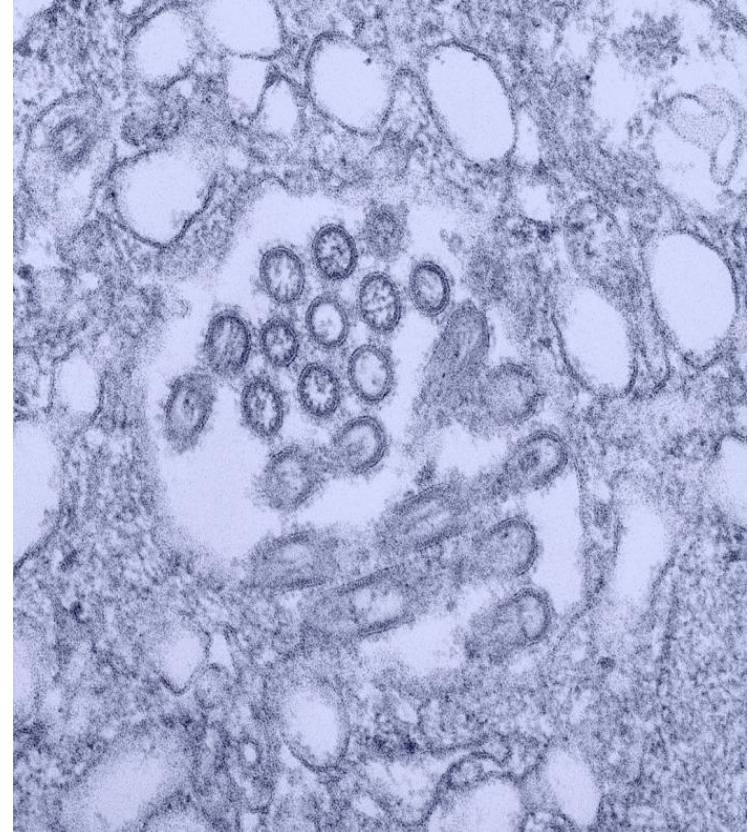
Western blot test:

- A test detects antimicrobial antibodies in the patient's sample (eg, serum, other body fluid) by their reaction with target antigens (eg, viral components) that have been immobilized onto a membrane by blotting.



Transmission Electron Microscopy (TEM)

- Specialized type of microscopy that utilizes a beam of electrons focused with a magnetic field to image a sample.
- TEM provides imaging with 1000x greater spatial resolution than a light microscope



Why diagnosis is important??

- Physicians should not request a report of "everything that grows," specific test.
- Irrelevant information could lead to inaccurate diagnosis and inappropriate therapy.
- Avoid specimen contamination," false positive".

- Specimens should be collected before starting antibiotics to avoid compromising culture results
- Laboratory test results should be accurate, significant, and clinically relevant.
- Complete and accurate specimen labelling is required (eg, dog bite wound, right forefinger).

Study resources

- Mim's Medical microbiology WW.CDC.gov.
- Medscape.com.
- WHO.int.org

Self assess

Discuss the different serological methods for diagnosis of viral infections?

Differentiate between the direct and indirect immunofluorescence tools?

Discuss the Immunologic Tests for Infectious Disease?

Questions

- 1.** When the agglutination reaction is used for detection of antibodies?
- 2.** What is the difference between the direct and indirect haemagglutination?
- 3.** What is the difference between the agglutination and the TEM?
- 4.** When are immunoelectrophoresis and countercurrent immunoelectrophoresis used?
- 5.** Write shortly the general principle of immunofluorescent assay & their types?

Thank you

Question ?

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